of Y263 were not clear. In this study, using classical molecular dynamics (MD) simulations and bias-exchange metadynamics, we identified a candidate ion-retaining state of the transporter (Bisha et al, J Chem Theor Comput, 2013). Furthermore, we found that the interplay between the two ligands is based on a weakly coupled mechanism in which the role of Y263 seems to be not relevant for the exiting of the galactose towards the cytoplasm.

1845-Pos Board B575

Coupling of Ion Binding and Conformational Equilibrium in Na^+-Driven Secondary Active Transporters

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In secondary active transporters, the electrochemical potential of ions across the membrane is used to fuel the ''uphill'' translocation of the substrate via the alternating access mechanism. The mechanism of this crucial coupling, however, remains unclear, despite significant recent experimental and computational studies. Mhp1, Na^+/Benzyl-hydantoin transporter, has become a key model for the secondary active transporters sharing the LeuT-fold topology. In the present study, we employed molecular dynamics (MD) simulations to study the impact of Na^+-binding on the structure and dynamics of Mhp1 in multiple functional states and on the transition between them. Microsecond-long equilibrium MD simulations suggest that Na^+ binding stabilizes the substrate-binding conformation in the outward-facing (OF) state, thereby conferring high affinity for substrate binding. Furthermore, the results of a special-protocol time-dependent biased simulation and subsequent free energy calculation for state transition, illustrate that Na^+ binding can increase the free energy barrier along the OF-IF transition. All the results suggest that cation binding reshapes the free-energy landscape of the ion/protein complex, thereby shifting the conformational preference toward a specific OF structure, which is favorable for substrate-binding. The increased substrate affinity provided by Na^+ binding will facilitate capturing the substrate from its low-concentration environment by the transporter. The results, therefore, provide a deeper and more comprehensive understanding for the ion-coupling mechanism of secondary active transporters.

1846-Pos Board B576

Mechanism of Sodium/Proton Antiport in NhaA

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The transmembrane protein NhaA from Escherichia coli is a prototypical sodium/proton antiporter. It enables the bacterium to grow under high salt conditions while homologous proteins in eukaryotes are involved in pH and cell volume regulation. A number of acidic and basic residues have been shown to be essential for the transport of one sodium ion for two protons but the mechanistic details of their involvement have not been fully determined. Furthermore, the conformational changes involved in the transport mechanism were not known. We present an unpublished crystal structure of NhaA in the inward facing conformation and of the homolog NapA in the outward facing conformation [1]. Using modelling and computer simulations we show how NhaA can function according to the alternating access model, resulting in large relative domain motions that are incompatible with previous structural models for transport in NhaA. Our structure of NhaA contains a salt bridge between the two conserved residues Asp163 and Lys300. With the help of molecular dynamics simulations we critically examine competing models for the molecular mechanism of the stoichiometric transport of two protons for one sodium ion, including one in which Lys300 maintains an active role in proton transport.

[1] Lee et al, Nature 501 (2013), 573.

1847-Pos Board B577

Functional Evaluation of NHE6 Mutation Associated with Syndromic Autism and Tau Deposition

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Mutations in the endosomal Na^+/H^+ exchanger NHE6 (*SLC9A6*) are associated with autism co-morbid with epilepsy and severe X-linked intellectual disability. In this work, using evolutionary conservation analysis, we built a model-structure of NHE6 based on the crystal structures of bacterial NhaA and NapA and used it to predict functional consequences of NHE6 mutations associated with autism and tau deposition. Based on this analysis we located the patient mutation p.Trp338_Thr340del in NHE6, corresponding to TM helix VII in NhaA. We showed that NHE6 transports protons out of the endosomal lumen to regulate trafficking of the amyloid precursor protein and processing to Abeta. We also studied the effect of normal and mutant NHE6 on tau aggregation and toxicity using a cell culture model of inducible tau expression. Taken together, these studies will advance our understanding of the mechanistic link between NHE6 and the trafficking and processing of endosomal cargo in neurons and glia and will provide insight into the molecular pathophysiology of autism and related disorders.

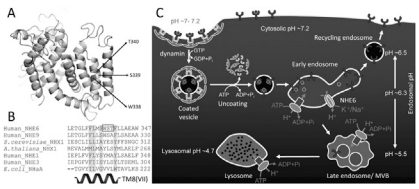

Figure: Model of NHE6 (**A**) depicting amino acid residues (**B**) deleted in syndromic autism. **C:** NHE6 regulates endosomal pH in vesicle trafficking.

1848-Pos Board B578

The Aspartate Transporter in Motion - Combining Steered Molecular Dynamics with Lanthanide Resonance Energy Transfer based Distance Measurements

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Excitatory amino acid transporters (EAAT's) or glutamate transporters belong to the SLC1 family of the neurotransmitter transporters and mediate the re-uptake of glutamate from the synaptic cleft. Crystal structures of GltPh, an archeal homologue of the mammalian glutamate transporter, have been solved in several states, providing a starting point for understanding the conformational changes that accompany substrate transport. In this study we aim to integrate molecular dynamics simulations and Lanthanide Resonance Energy Transfer (LRET) based distance measurements to study the molecular motions that accompany substrate transport. Steered Molecular Dynamics (SMD) simulations were used to obtain insights into the transition path that lead to internalization of substrate from the extracellular milieu. Our simulations revealed the existence of an intermediate state along the transition path from the outward-occluded to the inward-occluded conformation. Our simulations highlighted the existence of gatekeeper interactions at the transition from the intermediate state to the inward-facing state. Based on the dynamics observed from our simulations, cysteine mutants were designed to observe the conformational changes in vitro. Site directed mutagenesis was used to insert genetically encoded lanthanide binding tags (LBT) and also cysteines which act as fluorophore docking sites to perform LRET based distance measurements, thus generated LBT mutants were expressed and purified. The wild type and mutant proteins were expressed and purified using affinity column chromatography, donor decay signals were recorded for LBT insertion mutants to confirm the insertion of tags. Furthermore radioligand binding assays were performed with the mutants and they were found to be functional. The distance measurements made with LRET were compatible with the distances observed in the crystal structure.

1849-Pos Board B579

Transition Metal FRET to Study Conformational Changes in Glutamate Transporter

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Crystal structures of the bacterial glutamate transporter homologue GltPh developed show a series of different states of the transporter: an outward state with HP2 open, an outward state with HP2 closed, an inward occluded state and a recently reported intermediate conformation between outward and inward state. These crystal structures suggest that glutamate transporters undergo a series of conformational changes during substrate binding and transport. Previously we have used classical FRET methods to measure these conformational changes in human glutamate transporters EAAT3 during the glutamate transport cycle. But due to the long R_0 value of most FRET pairs, their large sizes and long flexible linkers, classical FRET methods are not always well-suited for mapping intramolecular movements in proteins. Here we used a new transition metal ion FRET method, which enables us to measure distance change within a very short range to better study the conformation change during the transport cycle. By labeling the FRET donor

fluorophore and the transition metal ion acceptor at different positions of glutamate transporter EAAT3, we will be able to measure intrasubunit distance changes during Na^+, glutamate binding and transport.

1850-Pos Board B580

Ionic Locks in Melibiose Permease: Scope towards GLUT1 Deficiency Syndrome

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The recently solved 3D crystal structures reveal that the Na^+-coupled melibiose permease of *Salmonella typhimurium* ($MelB_{St}$) is a member of the Major Facilitator Superfamily (MFS). These inside-closed conformations show that Arg residues at positions 295 (Helix IX), 141 (Helix V), and 363 ($Loop_{10-11}$) govern three clusters of electrostatic interactions for stabilizing the sealed internal cavity between N- and C-terminal domains at the cytoplasmic side. The cluster of these electrostatic interactions is designated as ionic lock. Except Lys at position 141 or 363, the replacement of R295, R141, or R363 individually with Cys, Gln, Glu, Leu, or Lys shows inhibition of active transport of melibiose to a level of 2 - 25 % of the WT, with little effect on the binding affinities for both sugar and Na^+. Interestingly, a suppressor D35E mutation at the periplasmic end of helix I was spontaneously isolated from the R363Q mutant. Remarkably, introduction of D35E mutation with each conformation-compromised mutant of R295, R141 (except R141E), or R363 significantly rescues the melibiose transport to a level up to 90 % of the WT. However, D35E mutation fails to restore the activity if two lock sites are mutated by Cys simultaneously at any combination. Previous threading model of $MelB_{St}$ at inside-open conformation show that all the three lock sites are at unlocked state. We conclude that these ionic locks are cooperative and involved in the transport-required global conformational changes by an alteration of locking and unlocking processes. Strikingly, these ionic locks are conserved in MFS permeases. Furthermore, they link with human diseases; e.g., some patients with GLUT1 Deficiency Syndrome are due to a single mutation of Arg at predicted ionic locks of the glucose transporter GLUT1 expressed in the blood-brain barrier.

1851-Pos Board B581

Functional Inhibition of Sugar Transport by a Designed Novel Protein

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Designed ankyrin repeat proteins have been shown to be useful for different applications such as structural, mechanistic, clinical studies, as well as drug development. We have constructed a combinatorial DNA library encoding ankyrin repeat proteins by PCR-based assembly. The ankyrin proteins have both N- and C-terminal caps and five internal repeats (called N5C). The estimated diversity of the library is higher than 10^{20}. Applying circular dichroism spectroscopy, four purified proteins exhibit high melting temperature (> 95 °C). We have tested the constructed library for the selection of specific ankyrin proteins targeting the melibiose transport in *Escherichia coli*. The melibiose permease is one of few overexpressed proteins during bacterial infection for gaining energy. Using cells with plasmid-encoded constructed library and chromosomally-encoded MelA, the alpha-galactosidase, and MelB, the melibiose permease, we have collected a group of N5C candidates that alter the melibiose fermentation with no inhibition of glucose fermentation, indicating no effect on the downstream utilization pathways. Three candidates show significant inhibition of melibiose transport. Further tests, using sugar transport assay on different genetic backgrounds, MelA activity assay, and protein-protein interaction assay, have been applied to identify specific effect. These preliminary studies suggested that one N5C protein possibly inhibits *mel* operon. These bio-reagents could be potentially applied to study the sugar transport during bacterial infection and also facilitate transporter proteins crystallography.

1852-Pos Board B582

An Insight into the XylE Transport Cycle by Characterizing Proton Binding Site and its Coupling to the Substrate Transport

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Characterizing the proton binding site and its coupling to the substrate transport??in XylE?

The diffusion of glucose across the plasma membrane is facilitated by carrier proteins called GLUTs. In the absence of a crystal structure for GLUTs, XylE serves as an excellent model to study the transport cycle in these proteins, not only due to its high sequence and structural similarity, but since it is the only MFS member with structures in various conformational states. In this study, we aim to characterize the structural determinants of substrate and proton binding and their coupling to protein conformational transitions in XylE. Using multiple sets of equilibrium MD simulations and free energy perturbation (FEP) calculations, we propose one of the acidic residues of XylE within the transmembrane region of the protein as the uncharacterized proton-binding site. Through comparative simulation studies, we show that the protonation of this residue results in the breakage of a salt bridge, which in turn induces the transition of the protein from the occluded state towards the open state. The observed conformational coupling, which was reproduced in multiple independent simulations, appears to involve the rotation of TM2 and TM7, and thereby perturbing the Xylose binding site as evident by the movement of Y298. Using sequence analysis of GLUTs we show the absence of this mechanistically important salt bridge in GLUTs, which can account for their H^+-independent transport mechanism. This work hence claims to give insight into the thermodynamic cycle corresponding to the change from outward facing occluded state to the open state and hence helps in elucidating the entire cycle in future.

1853-Pos Board B583

Mechanism of Tonb-Dependent Outer Membrane Transporters in E. Coli Mini-Cells and Outer Membrane Vesicles

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Current mechanistic models of the bacterial outer membrane TonB-dependent transporters (TBDT) suggest that transport is driven by the proton motive force (pmf) via interactions with the cytoplasmic membrane proteins TonB/ExbB/ExbD. Studies of these systems are challenging because of the lack of an in vitro reconstituted system, and transport must be studied in whole cells, which greatly limits biochemical and spectroscopic approaches. Use of evolved tRNA-tRNA synthetase pairs (Young et al.) along with orthogonal chemistry for specific placement of labels at non-sense mutations allows incorporation of labels in vivo. To enhance the specificity of the labeling, we developed the use of E. coli minicells that lack the chromosome but contain multi-copy plasmids. Only plasmid-encoded proteins are produced in minicells after separation from the mother cell and higher specificity of labeling is achieved. Moreover, minicells have the native arrangement and components of both membranes and periplasm, and physiological measurements can be made such as pmf-dependent uptake of vitamin B12 through the TBDT BtuB.

To study properties of the outer membrane transporter in the absence of the inner membrane, naturally occurring outer membrane vesicles (OMVs) (Schwechheimer et al.) are amenable for transport and spectroscopic studies. Both sides of the OMV membrane are accessible to labels and spectroscopic probes are incorporated into cysteines in over-expressed outer membrane proteins with high specificity. This work is supported by NIH/NIGMS grant U54 GM087519.

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1854-Pos Board B584

Alternating-Access Mechanism of the Proton-Drug Antiporter AcrB

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The AcrA/AcrB/TolC multidrug efflux pump confers Escherichia coli with antibiotic resistance by sequestering toxic compounds found within the periplasm and inner membrane and extruding them into the extracellular space. The AcrB trimer is the central component of this efflux complex. Anchored in the inner membrane, it forms an asymmetric assembly that undergoes a conformational cycle in which each protomer adopts three different structures (L, T and O states). Importantly, the conformational cycle within AcrB is driven by the translocation of protons down the electrochemical gradient sustained by the inner membrane, through a mechanism that has not been characterized so far. Here, we investigate this microscopic mechanism through structural modeling, electrostatic calculations and molecular dynamics simulations based upon novel high-resolution structural data.

Our results show how reversible protonation of key sites within the transmembrane domain translate into structural changes that are transduced to the periplasmic domain, thus coupling proton and drug transport. These conformational changes can be understood in terms of semirigid roto-translations of two